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B1X X16 X8

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(58) Field of Search

UK CL (Edition O) C2C CMA
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ONLINE: CAS-ONLINE, EDOC, JAPIO, WPI

(54) COMBINATORIAL PREPARATIVE PROCESS USING ELECTROPHORESIS

(57) A combinational process is carried out in a μ -FFE microstructure which has a bed (315) isolated from the electrodes in beds (308 and 309). Reactants A and B are fed into bed (315) which acts as a reaction zone; a differential voltage field is then applied across the bed and unreacted material and product are separated by electrophoresis.

The apparatus issued to prepare 4-(phenylazo)-phenol from a diazonium salt and phenol.

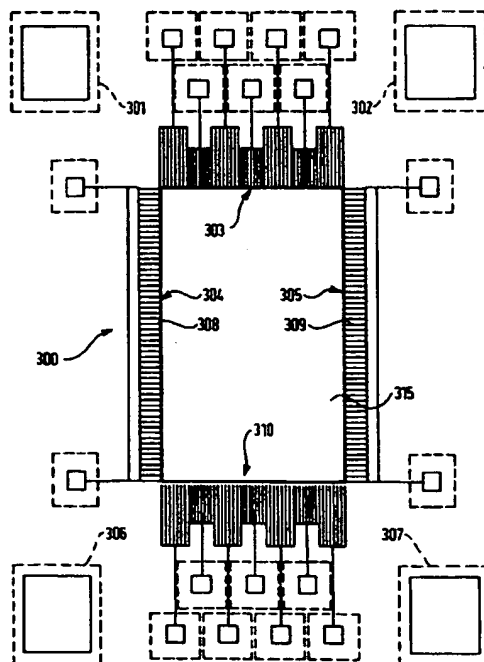


FIG. 7

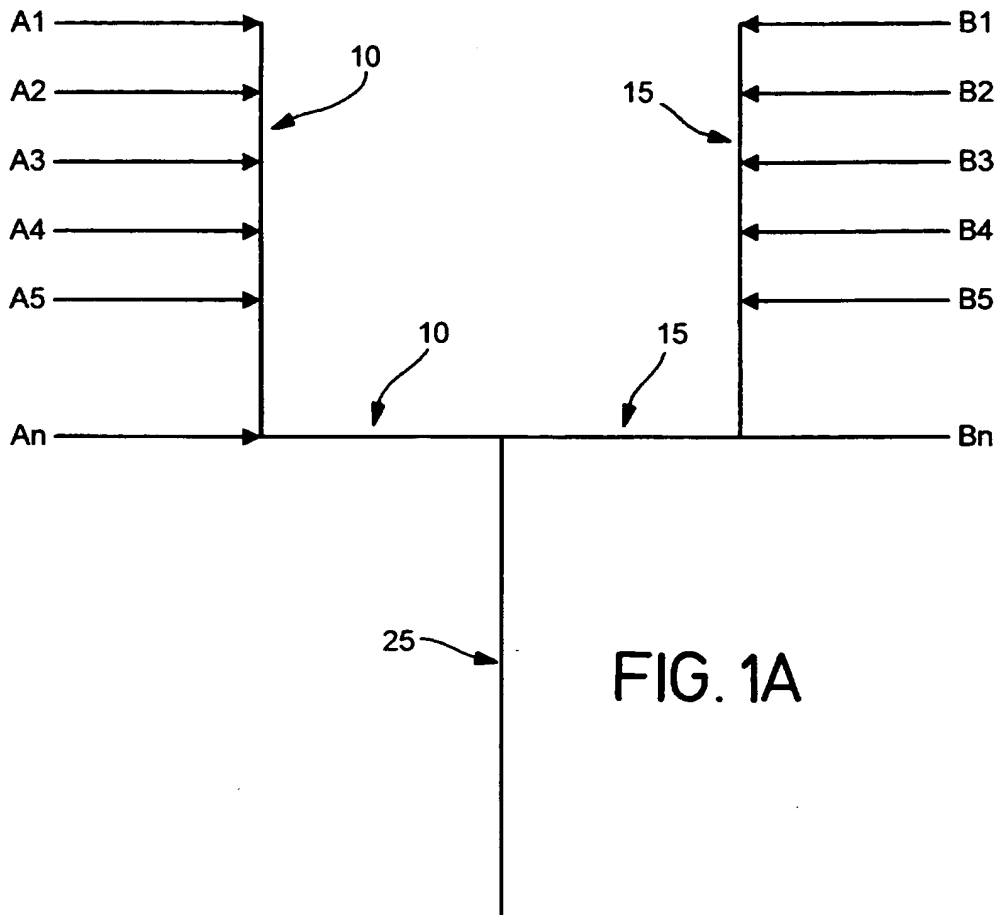


FIG. 1A

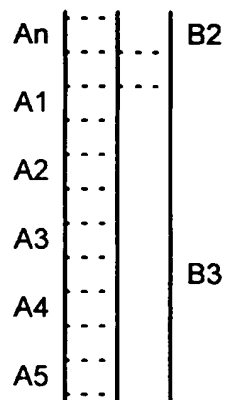


FIG. 1B

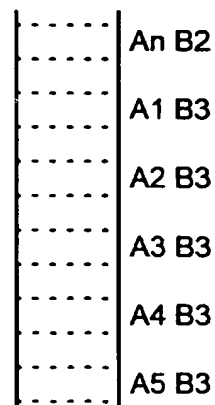


FIG. 1C

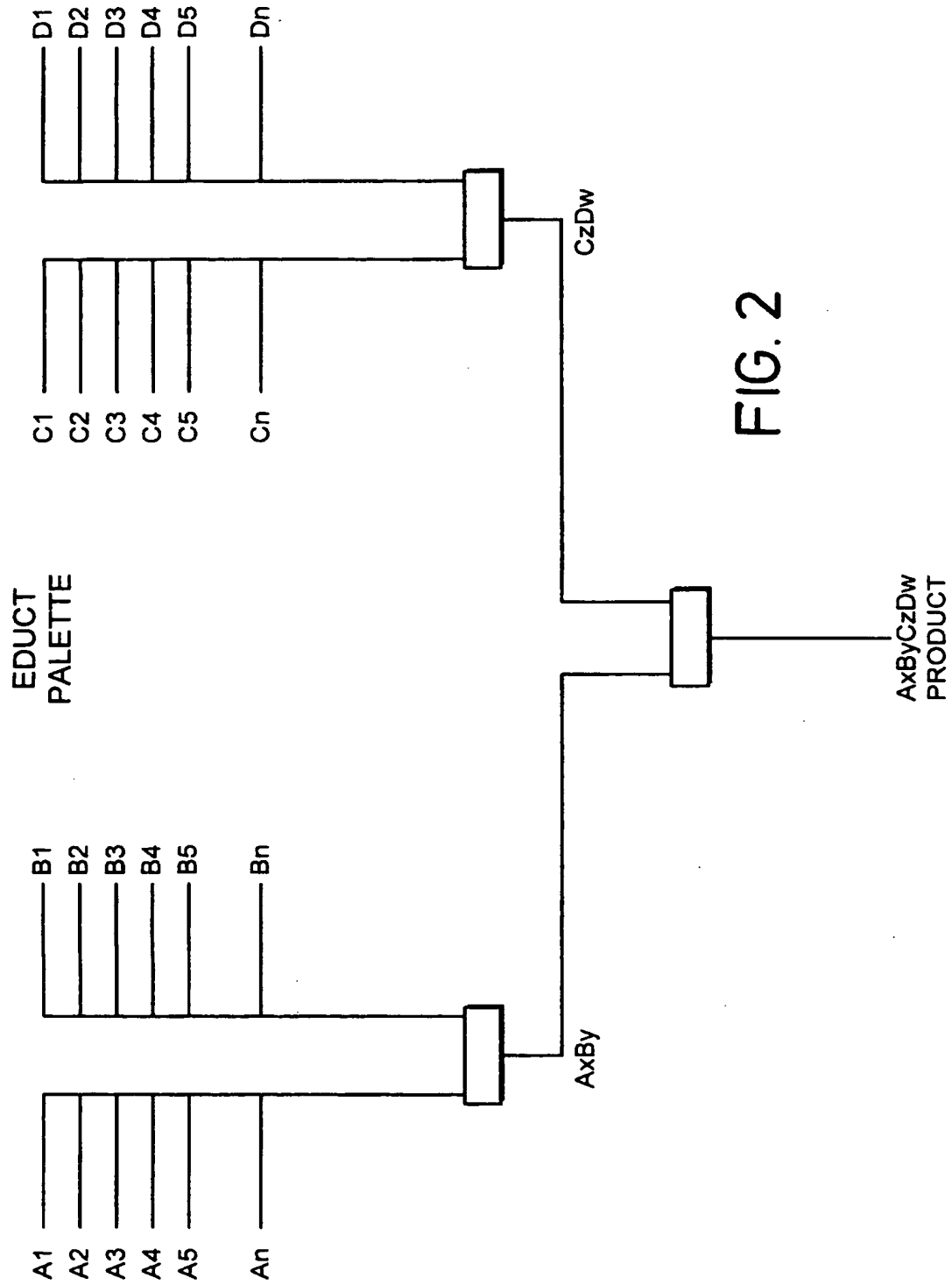
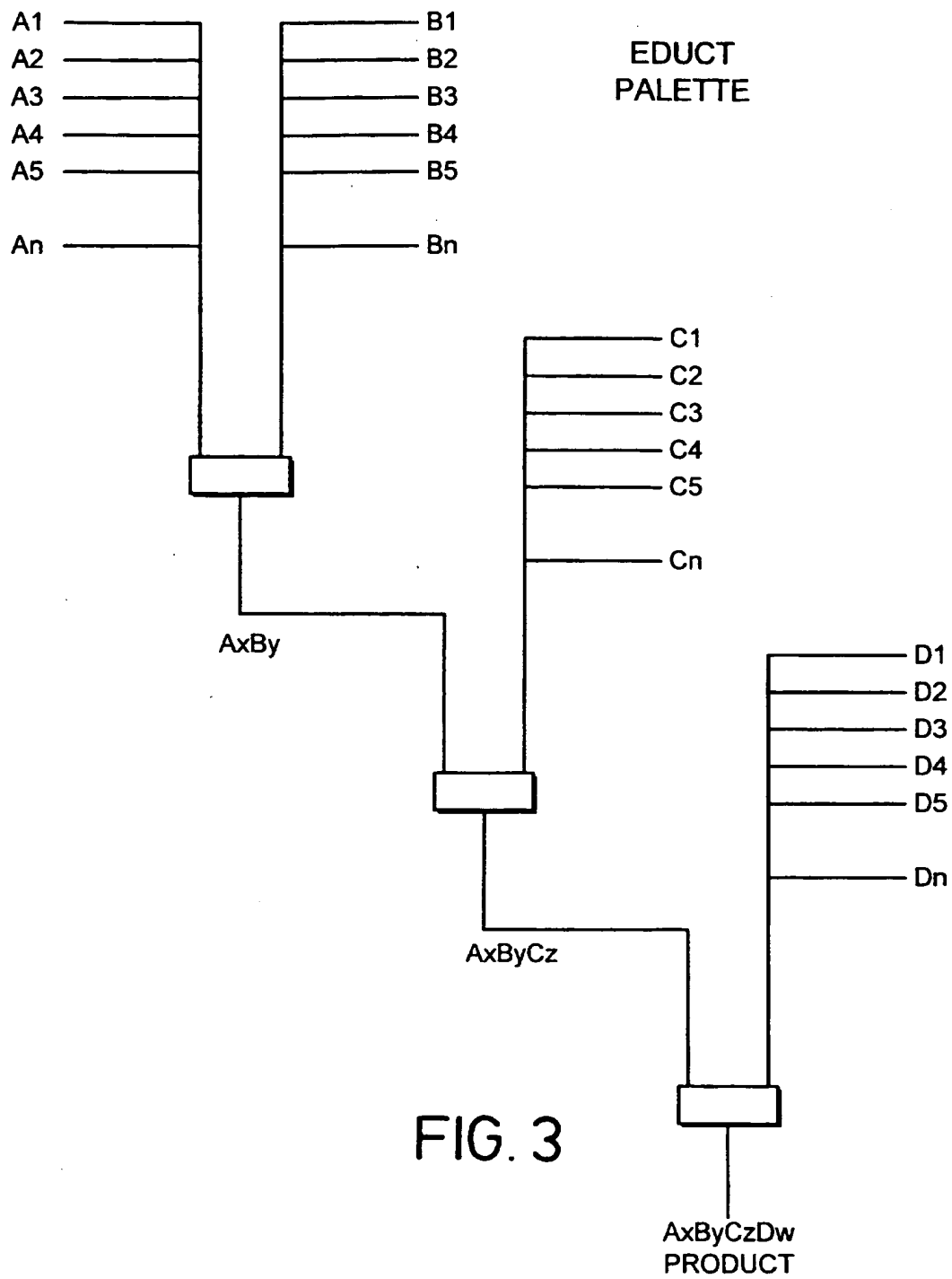


FIG. 2



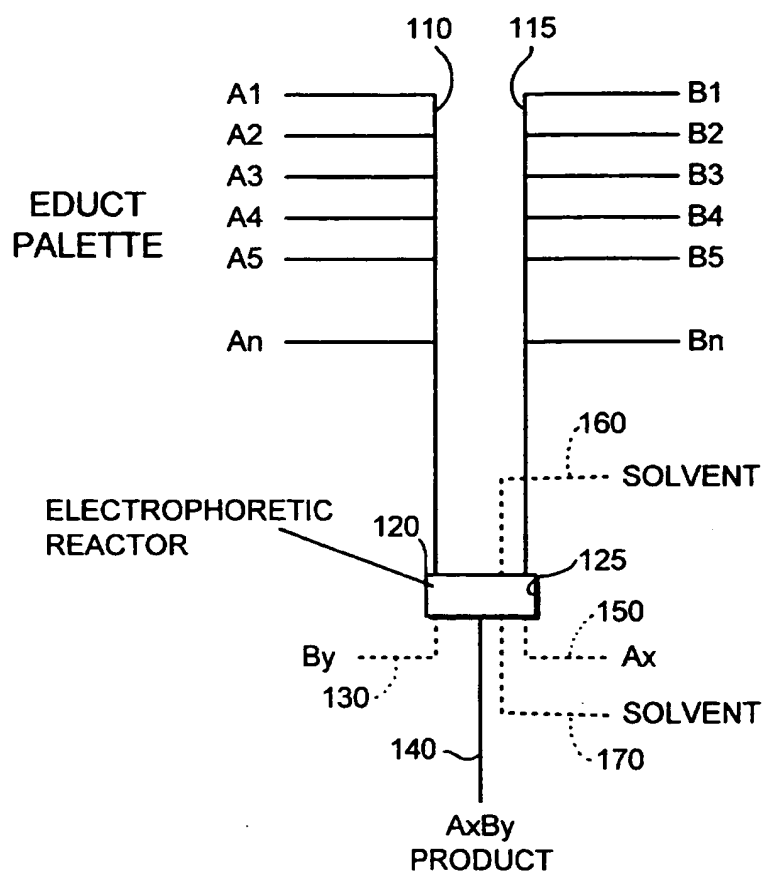


FIG. 4

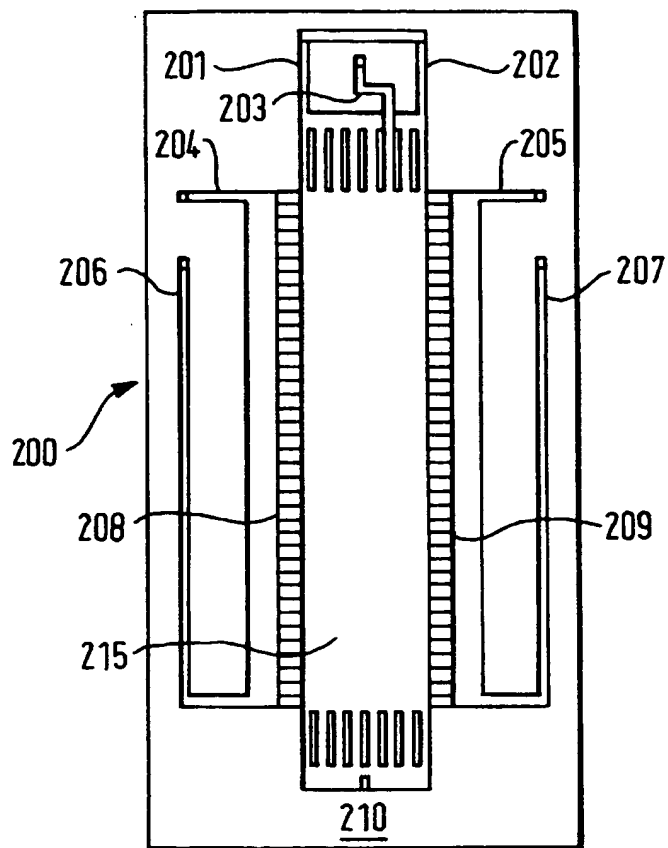


FIG. 5

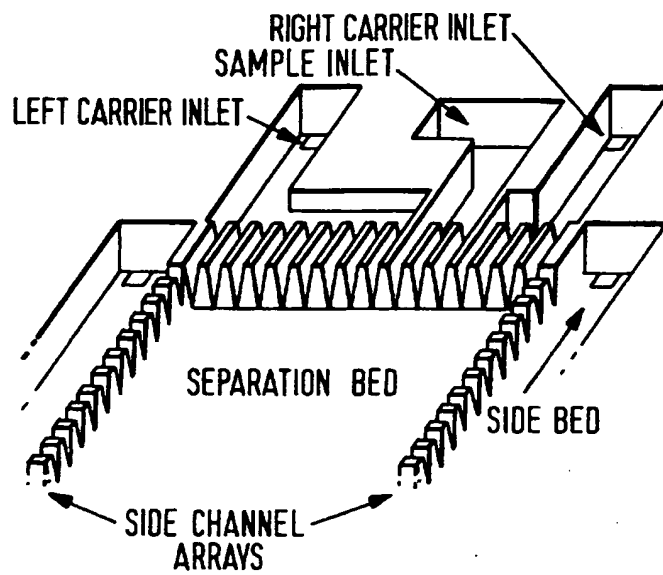


FIG. 6

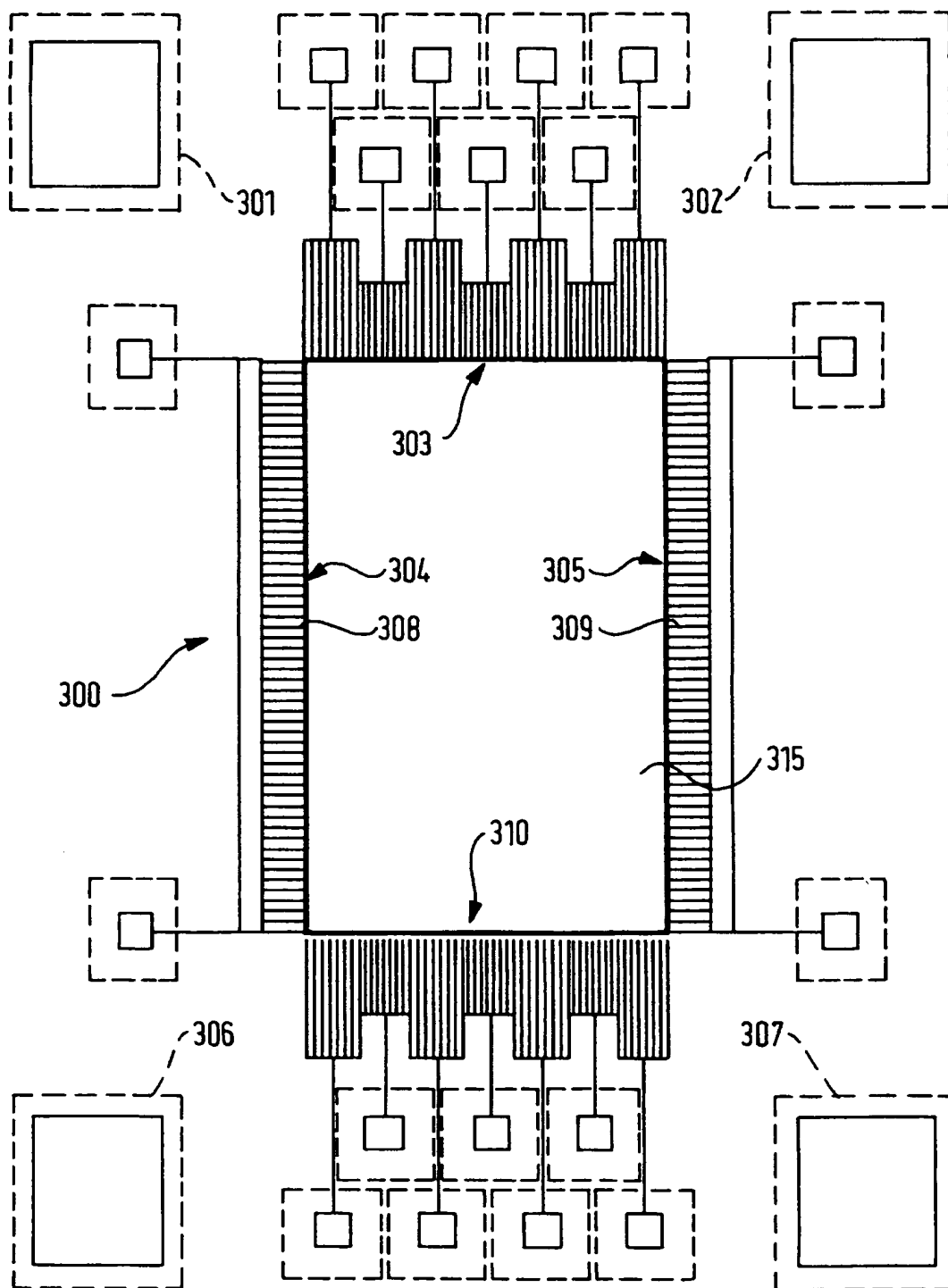


FIG. 7

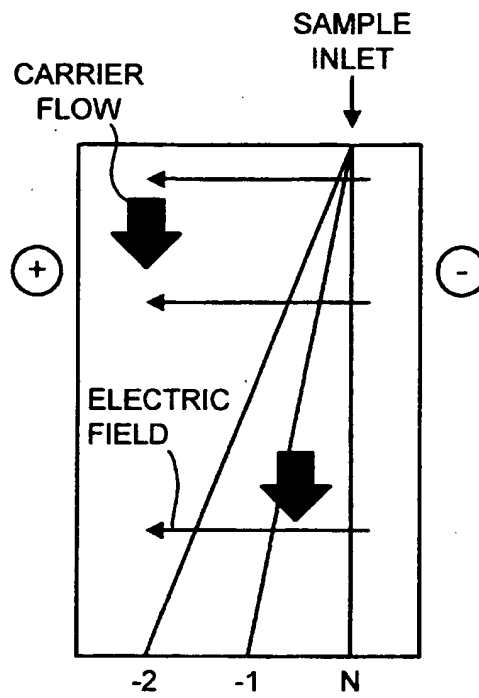


FIG. 8

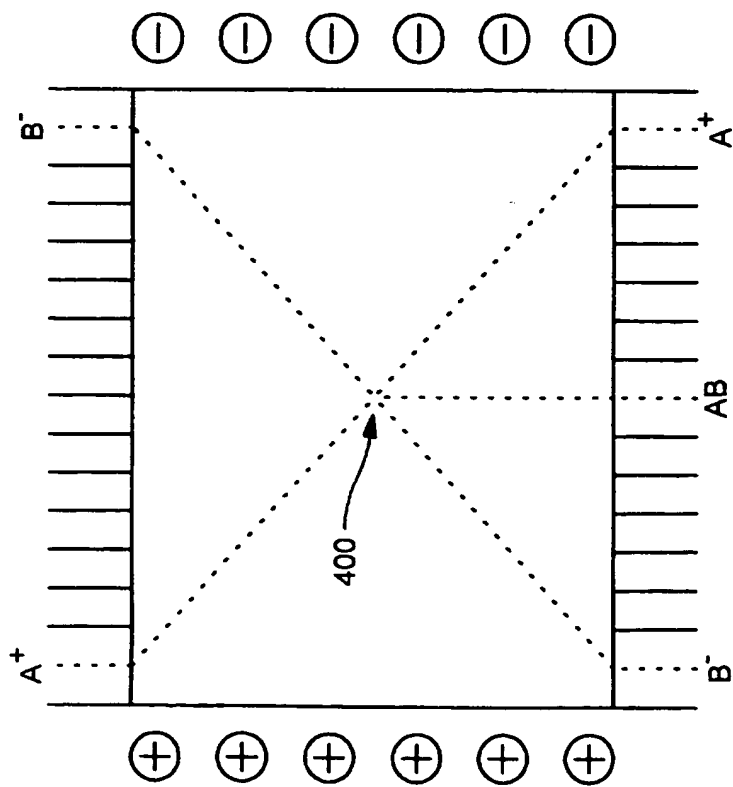


FIG. 9

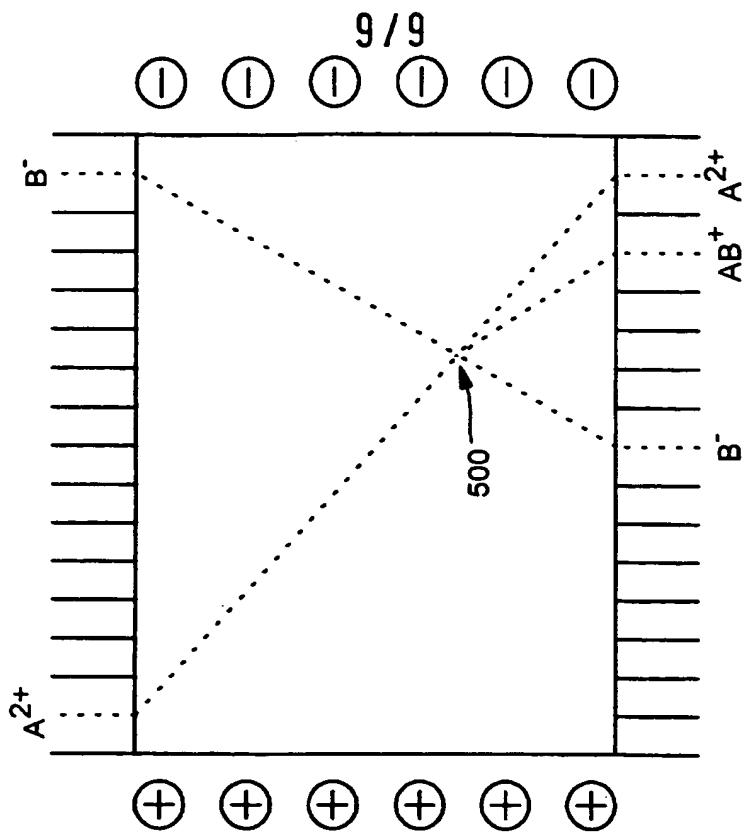


FIG. 10

PROCESS

The present invention relates to a process. In addition, the present invention provides a device suitable for use in that process.

In particular the present invention provides a process suitable for use in combinatorial chemistry.

Combinatorial chemistry is a valuable tool for synthetic chemistry. It allows extremely large numbers of compounds - otherwise known as a library of compounds (or products) - to be synthesised in a reasonable time frame. In brief, the compounds are synthesised by a multi-step wise addition of materials (such as molecules) to another material (such as another molecule) or other materials (such as other molecules) that have been already linked together. The synthesised compounds can then be screened to see if, for example, they show promising favourable biological activity.

Introductory reviews on combinatorial chemistry can be found in the teachings of Gallop *et al* (*J Med Chem* 1994, 37, 1233) and Gordon *et al* (*J Med Chem* 1994, 37, 1385).

The application of combinatorial chemistry has led to the preparation of libraries of different benzodiazepines (WO95/02566), phenolic derivatives (Leznoff *Acc Chem Res* 1978, 11, 327-333), endothelin antagonists (Bunin *et al* PNAS 1994, 91, 4708-4712) and α 1-adrenergic antagonists (Furka *et al*, *Int J Peptide Protein Res*, 1991, 37, 487-493).

One problem with combinatorial chemistry is the isolation of products from the synthetic reaction medium or media. To solve this problem, Szymonifka and Chapman (*Tetrahedron Letters*, 1995, vol 36, No. 10, pp1597-1600) proposed the use of magnetically manipulable polymeric supports for solid phase organic combinatorial synthesis of products.

According to Szymonifka and Chapman (*ibid*) "Typically, in combinatorial organic synthesis, an appropriately functionalized solid phase support is partitioned among a number of reactors and unique subunits are reacted with each pool of resin in each reactor. The resin beads are then removed entirely from all reactors, combined and mixed until homogeneous. This mixture is then redistributed to the reactors and the process is repeated, usually with a different set of unique subunits. If there are no redundancies in the subunits, the number of compounds produced by such a scheme is the number of reactors expanded to the exponent of the number of steps in which subunits are coupled. Thus, three sequential reactions using twenty pools of resin and unique subunits affords 20^3 products."

To date, most combinatorial chemical processes are solid state processes. In addition to the use of beads, plates having thereon spaced-apart, immobilised reactants may be used. The immobilised reactants are reacted, in a step-wise fashion, with further reactants. After a series of reactions a wide range of compounds are prepared. Each of these compounds is effectively space-encoded on the plate as the location of the original starting reagents are known. Knowing the x and y spatial coordinates of the original starting reagents - and hence of each resultant compound - allows each synthesised compound to be identified for screening or analysis.

Even though the known combinatorial chemical processes allow workers to prepare libraries of compounds, they do suffer from the draw back that they are dependent on solid state chemical reactions which limits, to some extent, the type of reactions that can be performed and ultimately the resultant products that can be made. For example, some reactant molecules are not suited to solid phase reactions, especially small molecules. Also, many reactions have to be adapted to suit the solid phase reaction environment and this can be problematical in itself and, in some cases, is not possible.

Also, with the solid phase reactions the reactant molecules and reaction products have to be identified by means such as spectroscopy or by tagging, each of which is an additional step that is laborious, expensive and timely.

Furthermore, the solid phase reactions are not suitable for the use of robotic or fluid handling. For example, the reactions are too slow to devise an acceptable robotic synthesis scheme. This is a significant drawback as it means that the solid phase reactions are, for some applications, too slow.

5

In addition, typically the overall combinatorial chemical process comprises two or more of synthesis steps, separation steps, screening steps and analysis steps. In the known combinatorial chemical processes there can be a delay that overall process. Typically the analysis steps are much slower than the synthesis steps - which can result in an unacceptable back log.

10

The present invention seeks to overcome one or more of the problems associated with the known combinatorial chemical processes. In particular, the present invention seeks to improve upon the known combinatorial chemical processes.

15

According to a first aspect of the present invention there is provided a multi-step process comprising the sequential steps of:

- (a) individually introducing reactant A1 and reactant B1 into a reaction zone R1;
- 20 (b) allowing A1 and B1 to contact each other in the reaction zone R1;
- (c) individually removing from the reaction zone R1 any product P1 formed by the reaction of A1 with B1, any unreacted A1 and any unreacted B1;
- (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2;
- 25 (e) allowing A2 and B2 to contact each other in the reaction zone R2;
- (f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2;
- 30 wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time; and
- wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time.

According to a second aspect of the present invention there is provided a device for a multi-step process, which process comprises the sequential steps of:

- (a) individually introducing reactant A1 and reactant B1 into a reaction zone R1;
- 5 (b) allowing A1 and B1 to contact each other in the reaction zone R1;
- (c) individually removing from the reaction zone R1 any product P1 formed by the reaction of A1 with B1, any unreacted A1 and any unreacted B1;
- 10 (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2;
- (e) allowing A2 and B2 to contact each other in the reaction zone R2;
- (f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2;
- 15 wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time; and
- wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time;

wherein the device comprises

- 20 (i) a region providing reaction zone R1;
- (ii) a region providing reaction zone R2;
- (iii) an inlet for reactant A1 and an optional separate inlet for reactant A2;
- (iv) an inlet for reactant B1 and an optional separate inlet for reactant B2;
- 25 (v) an outlet for unreacted reactant A1 and an optional separate outlet for unreacted reactant A2;
- (vi) an outlet for unreacted reactant B1 and an optional separate outlet for unreacted reactant B2; and
- 30 (vii) an outlet for any product P1 and an optional separate outlet for product P2;

wherein the inlets and outlets are operably connected to the reaction zones.

According to a third aspect of the present invention there is provided a time-encoded, sequential, multi-step, chemical reaction process.

5 According to a fourth aspect of the present invention there is provided a method of performing combinatorial chemistry in which reactants are introduced in to a reaction zone by means of a charge field, and the products of the reaction are subjected to a charge field to separate the products.

10 According to a fifth aspect of the present invention there is provided a compound when synthesised, analysed or screened by the multi-step process according to the present invention.

15 The process of the present invention can be any chemical process, such as a synthetic process, a degradative process, an analytical process, an activity assay, a binding assay etc.

20 One of the key advantages associated with the present invention is that it enables a library of compounds to be prepared or screened by chemical reactions that are not dependent on the limitations of solid state chemistry. In particular, the present invention enables a library of compounds to be prepared by liquid (e.g. solution) state chemistry, which offers distinct advantages over just solid state chemical reactions.

25 The present invention also overcomes one or more of the above-mentioned inherent problems associated with the known solid phase combinatorial chemical reactions. For example, the present invention is suitable for the reaction of small molecules. The present invention is also suited to robotic or fluid handling. Furthermore, as the reactants are added in a set time-wise fashion so the reactions are time encoded. This means that there is less (or even no) requirement to identify the resultant products by means such as spectroscopy or by tagging.

30

Another key advantage of the present invention is that it facilitates the integration of any one or more of the following combinations: synthesis and separation; analysis and separation; screening and separation; synthesis and analysis and separation; synthesis and screening and separation; synthesis and analysis and screening and separation.

5 Hence, the process of the present invention can speed up the overall combinatorial chemical process - thereby enabling compounds to be synthesised, separated, screened and analysed faster.

10 As already mentioned, one of the key advantages of the present invention is that it allows time encoded reactions to take place. In this regard, knowledge of the time of addition of one or more of the reactants provides a means for separating and identifying the possible composition of the resultant product.

15 Another key advantage of the present invention is that it allows a series of sequential reactions to occur simultaneously - otherwise known as multiplexing.

20 Another key advantage of the present invention is that it is very well suited to allowing sequential rapid reactions to occur in one reactor. Likewise, the present invention is very well suited to allowing sequential rapid reactions to occur in parallel for an even higher output.

25 The term "individually introducing" as used herein includes separate, simultaneous introduction. The term also includes sequential introduction, such as from different inlets. Preferably the term means separate, simultaneous introduction from different inlets.

The term "individually removing" as used herein includes separate, simultaneous removal. The term also includes sequential removal, such as from different outlets. Preferably the term means separate, simultaneous removal from different outlets.

30

The term "at a predetermined time" as used herein also includes at a time after a predetermined time period.

The term "charge" as used herein includes neutral charge, as well as positive charge and negative charge.

5 The term "wafer device" as used herein means a substantially flat, three dimensional structure, preferably of a small size. Preferred sizes are as follows:- length: 3 mm to 10 mm; width: 3 mm to 10 cm; and height (or thickness): 50 microns to 50 mm. A preferred wafer device is a silicon glass chip or is a polymeric chip.

10 The term "charge field" as used herein means any suitable charge field. Preferably the term means an electric charge field.

Reaction zone R1 and reaction zone R2 can be the same or different.

15 Reactant A1 and reactant A2 can be the same or different.

Reactant B1 and reactant B2 can be the same or different.

Reactant A2 and/or reactant B2 can be product P1.

20 The process may include one or more additional, subsequent reaction steps, such as the addition of one or more reactants A_n wherein n is greater than 2 - such as A3, A4, A5 etc. - into a reaction zone R_n wherein n is greater than 2 - such as R3, R4, R5 etc. - wherein each addition occurs at a predetermined time and/or the addition of one or more reactants B_n wherein n is greater than 2 - such as B3, B4, B5 etc. -
25 into the reaction zone R_n wherein each addition occurs at a predetermined time, thereby to possibly form additional products collectively called P_n wherein n is greater than 2 - such as P3, P4, P5 etc..

30 Reactant(s) A_n may be the same or different with respect to each other and/or with respect to reactant A1 and reactant A2.

Reactant(s) B_n may be the same or different with respect to each other and/or with respect to reactant B₁ and reactant B₂.

5 Likewise, reactant A_n and/or reactant B_n can be any one or more of product P_{n-1}, P_{n-2}, P_{n-3} etc.

10 The reaction that occurs in a particular reaction zone such as R₁, R₂ or R_n - collectively called R (which may be the same or different) - may be a synthetic step. In this case, for example, if A₁ were an acid and B₁ were an alcohol then P₁ would be an ester. In this case, the ester and any unreacted acid and alcohol would be individually removed from the reaction zone R₁.

15 The reaction that occurs in the reaction zone R may be an analytical step. In this case, for example, if A₁ were an acid and B₁ were a pH indicator capable of being converted to a coloured derivative in the presence of an acid then P₁ would be the coloured derivative. In this case, the colour change would indicate the presence of an acid. Also, the coloured derivative and any unreacted acid and pH indicator would be individually removed from the reaction zone R₁.

20 The reaction that occurs in the reaction zone R may be a screening step - such as screening for a favourable biological activity. In this case, for example, if A₁ were a possible DNA nicking agent and B₁ were a polynucleotide strand capable of being nicked then P₁ would be the nicked DNA strand and/or the fragments thereof. In this case, any nicked DNA strand or the fragments thereof, and any unreacted A₁ and B₁ would be individually removed from the reaction zone R₁. The presence of any nicked DNA strand or the fragments thereof would indicate that A₁ was a suitable DNA nicking agent.

30 The process may include the addition of one or more a number of additional reactants, collectively referred to as X_n wherein n is an integer - such as X₁, X₂, X₃ etc. - into a particular reaction zone R at a predetermined time wherein each addition occurs at a predetermined time thereby to possibly form more complex products

collectively referred to as CP wherein n is an integer - such as CP1, CP2, CP3, CP4, CP5 etc..

5 The process may even include the additional step(s) of introducing any one or more of: any one or more of unreacted A1, A2, An; any one or more of unreacted B1, B2, Bn; any one or more of unreacted X1, X2, Xn; any one or more of products P1, P2, Pn; and any one or more of products CP1, CP2, CPn, into one or more further reaction zones collectively referred to as FRn where n is an integer - such as FR1 or FR2 - for further subsequent reactions (such as synthesis and/or analysis and/or
10 screening) and separation.

For example the process of the present invention could comprise a first stage of synthesising a product P1 from A1 and B1 in a first reaction zone R1 and then individually separating P1 and any unreacted A1 and B1 from the reaction zone R1
15 (by the afore-mentioned steps of the process of the present invention) and then screening the product P1 by use of a substrate B2 in a further reaction zone FR1 and then individually separating P2 (i.e. any reaction product between P1 and B2) from the reaction zone FR1 (by the afore-mentioned steps of the process of the present invention).

20 Any suitable reactants for any one of A (i.e. any one of A1, A2, An), B (i.e. any one of B1, B2, Bn) and Xn may be used. As indicated above the reactants may be used in a synthetic step, or in an analytical step or in a screening step.

25 In one preferred embodiment reactant A1 and reactant A2 are different to each other. With this embodiment reactant B1 and reactant B2 may be the same or different to each other. If the process is a synthetic process then B1 and reactant B2 may be the same.

30 Likewise, in one preferred embodiment reactant B1 and reactant B2 are different to each other. With this embodiment reactant A1 and reactant A2 may be the same or different to each other. If the process is a synthetic process then A1 and reactant A2

may be the same.

5 In another preferred embodiment reactant A1 and reactant A2 are the same. With this embodiment reactant B1 and reactant B2 may be the same or different to each other. If the process is an analytical process or a screening process then B1 and reactant B2 may be different to each other.

10 Likewise, in another preferred embodiment reactant B1 and reactant B2 are the same. With this embodiment reactant A1 and reactant A2 may be the same or different to each other. If the process is an analytical process or a screening process then A1 and reactant A2 may be different to each other.

15 In a preferred embodiment at least one of reactants A and B is charged. Preferably each of reactants A and B is charged. Preferably the reactants A and B are of a different charge. More preferably reactants A and B are of opposite charge.

20 In a preferred embodiment, the product P (i.e. any one of P1, P2, Pn) is of a different charge to A and/or B. Preferably, the product P is of a different charge to A and B.

The possible reaction of reactant A with reactant B within the reaction zone can be aided or even commenced by additional physical factors - such as the application of any one or more of heat, cold, light and pressure.

25 It is also possible to vary the reaction environment to suit particular needs (such as optimisation) - such as adding particular buffers, solvents, solutions, catalysts, initiators, etc.

30 The products or reactions can be analysed by any suitable analytical technique - such as by the use of laser-induced fluorescence, or by off-line capillary electrophoresis such as when samples have been obtained *via* fraction collection.

One or more of the reactants may be pre-treated. In this regard, and by way of example, one or more of the reactants may be imparted with a charge group (or even an extra charge group) that could be cleaved off after the reaction step.

5 Preferably the reaction zones are located in or on a microliter volume free-flow electrophoresis microstructure, which is sometimes referred to as a " μ -FFE microstructure". A suitable μ -FFE microstructure is described in two papers of D. Raymond, A. Manz and H. Widmer - these being Analytical Chemistry Vol 66 No. 18 September 15 1994 pages 2858 to 2865 and Analytical Chemistry Vol 68 No. 15
10 August 1 1996 pages 2515 to 2522 - the contents of which are incorporated herein by reference.

Preferably, the process occurs in or on an integrated total chemical analysis system (otherwise referred to as "TAS") - such as on glass and/or silicon. These systems are
15 sometimes referred to as either "miniaturised TAS" or " μ -TAS". A recent review of μ -TAS by Caston and Cowen may be found in Chemistry in Britain (October 1996 edition, pages 31-33), the contents of which are incorporated herein by reference.

Each of reactants A, B and X may have their own separate inlets. Each of unreacted
20 reactants A, B and X may have their own separate outlets. Each of products P and CP may have their own separate outlets.

Preferably the process is a combinatorial chemical process.

25 Preferably the reactants A1 and/or A2 are charged.

Preferably the reactants A1 and A2 are charged.

Preferably the reactants B1 and/or B2 are charged.

30

Preferably the reactants B1 and B2 are charged.

Preferably the reaction zone is in or on a wafer device.

Preferably the wafer device is a μ -FFE microstructure.

5 Preferably the reactants are introduced into the reaction zone(s) by the use of a differential charge field.

Preferably the products and unreacted reactants are removed from the reaction zone(s) by the use of a differential charge field.

10

Preferably there is a separate inlet for reactant A2 and/or a separate inlet for reactant B2.

15

Preferably there is a separate outlet for unreacted reactant A2 and/or a separate outlet for unreacted reactant B2.

Preferably there is a separate outlet for product P2.

20

Preferably the device is a wafer device.

Preferably the wafer device is a μ -FFE microstructure.

Preferably the device comprises means for delivering a differential charge field in order to introduce the reactants into the reaction zone(s).

25

Preferably the device comprises means for delivering a differential charge field in order to remove the products and unreacted reactants from the reaction zone(s).

30

Preferably any of product P1 and unreacted reactant A1 and B1 are in or on the device and have left the reaction zone R1 and wherein reactants A2 and B2 are in or on the device and are about to enter the reaction zone R2.

Preferably the addition steps and reaction steps and removal steps in the multi-step process are sequential.

In order to explain even more fully the process of the present invention reference shall be made to the schematic diagrams shown in Figures 1 - 4.

In Figure 1, a series of reactants A - shown as A1, A2 etc - are time-wise reacted with a series of reactants B - shown as B1, B2 etc.

In the process, reactants A are individually fed into a first reaction zone (25) via an inlet (10) - such as from a reservoir (not shown) and by use of appropriate valve means (not shown) and feeder means (not shown). In this regard, the reactants A are fed in a time encoded manner - e.g. at certain time intervals, which may be the same or different - into the inlet (10) and thus into the reaction zone (25).

In the process, reactants B are individually fed into the first reaction zone (25) via an inlet (15) - such as from a reservoir (not shown) and by use of appropriate valve means (not shown) and feeder means (not shown). In this regard, the reactants B are also fed in a time encoded manner - e.g. at certain time intervals, which may be the same or different - into the inlet (15) and thus into the reaction zone (25).

A number of reactions are possible, each of which depends on the timing of the addition of reactants A and reactants B with respect to each other.

For example, and as shown in Figure 1B, if reactants A are fed into the reaction zone at a faster rate than reactants B then it is possible to have a first phase of spaced apart reactions wherein each different reactant A is reacted with the same reactant B followed by a second phase of spaced apart reactions wherein each different reactant A is reacted with the same reactant B but wherein reactant B in the second phase of reactions is different to reactant B in the first phase of reactions. It is then possible to have a third phase of spaced apart reactions wherein each different reactant A is reacted with the same reactant B but wherein reactant B in the third phase of reactions

is different to reactant B in the first phase and the second phase of reactions. Thus, the possible reaction products in the first phase of the spaced apart reactions are A1B1, A2B1, A3B1 etc; the possible reaction products in the second phase of the spaced apart reactions are A1B2, A2B2, A3B2 etc; and the possible reaction products in the third phase of the spaced apart reactions are A1B3, A2B3, A3B3 etc (as shown in Figures 1B and 1C). In each of the phases of reactions the reactants and their products are spaced apart as a result of the timing of addition of the reactants to the reaction zone. Hence, in the present invention the reactions are time encoded - rather than spatially encoded as is the case with the known combinatorial chemical processes that utilise immobilised reactants. The products and the unreacted reactants can then be individually removed from the reaction zone.

Figures 2 and 3 illustrate how it is possible to use the process of the present invention to prepare libraries of more complex compounds - such as for subsequent analysis or screening.

Thus, in its broadest sense the present invention provides a chemical process comprising a number of sequential chemical reaction steps wherein the reaction steps are time encoded.

A preferred embodiment of this broad aspect of the present invention is a combinatorial chemical process comprising a number of sequential chemical reaction steps wherein the reaction steps are time encoded.

Another preferred aspect of the present invention includes a method of performing combinatorial chemistry in which reactants (A1; A2 ...) of a first set of reactants (A) are introduced into a reaction zone one by one sequentially in time, and another reactant (B) is introduced into the reaction zone simultaneously with the reactants (A1 A2...) of the first set (An). Preferably, the said another reactant (B) is one reactant (B1) of a second set of reactants which are introduced into the reactor zone one by one sequentially in time.

Another preferred aspect of the present invention includes a method of performing combinatorial chemistry in which reactants (A) are charged with a first charge, reactants (B) are charged with a second charge different to the first charge (e.g. of opposite charge), and the reactants (A) and (B) are directed by means of a charge field into a reactor zone where they react. Preferably the charge field is an electric field.

Another aspect of the present invention includes a method of performing combinatorial chemistry in which reactants are introduced in to a reaction zone by means of a charge field, and the products of the reaction are subjected to a charge field to separate the products.

A preferred device for use with the present invention is an electrophoretic reactor. Figure 4 diagrammatically shows this preferred aspect of the present invention. Figure 4 shows an inlet (110) through which any one or more of reactants A can flow into an electrophoretic reactor (120) and into a reaction zone (125) located therein. Figure 4 also shows an inlet (115) through which any one or more of reactants B can flow into the electrophoretic reactor (120) and into the reaction zone (125) located therein. The timing of the addition of reactants A and B and the consequences thereof has been discussed above. Figure 4 also shows outlet (130) for any unreacted reactant A. Figure 4 also shows outlet (150) for any unreacted reactant B. Figure 4 also shows outlet (140) for any product A-B. Solvent inlet (160) and solvent outlet (170) allows suitable solvent(s) to flow through the reaction zone to aid *inter alia* the contacting, reacting and separation of the reactants and the products thereof.

As indicated above, a preferred electrophoretic reactor is a μ -FFE microstructure. A preferred μ -FFE microstructure is described in two papers of D. Raymond, A. Manz and H. Widmer (*ibid*) - the contents of which are incorporated herein by reference. These papers do not disclose or suggest the use of that device in the process of the present invention. In this regard, in these papers the device is only used for separation.

A cross-sectional view of such a μ -FFE microstructure is shown in Figure 5. In this regard, the microstructure (200) comprises a first carrier buffer inlet (201), a second carrier buffer inlet (202), a sample inlet (203), a first set of side bed inlets (204), a second set of side bed inlets (205), a first set of side bed outlets (206), a second set of side bed outlets (207), a first set of side bed containing platinum electrodes (208), a second set of side bed containing platinum electrodes (209), an outlet (210) and a bed (215).

An expanded and perspective view of part of the μ -FFE microstructure (200) is shown in Figure 6.

For the present invention, it is highly preferred that the inlet (203) comprises a series of spaced-apart inlet ports through which the reactants A and B can travel onto the bed (215).

In addition, for the present invention, it is highly preferred that the outlet (210) comprises a series of spaced-apart outlet ports through which any product P and any unreacted reactants A and B can travel away from the bed (215).

In addition, for the present invention, the bed (215) provides a reaction zone and a separation zone for the reacted and unreacted reactants.

A more suitable μ -FFE microstructure for combinatorial chemistry is shown in Figure 7, wherein the microstructure (300) comprises a set of access holes (301) for electrode bonding pads (not shown), a set of access holes (302) for electrode bonding pads (not shown), a series of spaced-apart sample inlets (303), a first set of side bed inlets (304), a second set of side bed inlets (305), a set of access holes (306) for electrode bonding pads (not shown), a set of access holes (307) for electrode bonding pads (not shown), a first side bed containing a platinum electrode (308), a second side bed containing a platinum electrode (309), a series of spaced-apart outlets (310) and a bed (315). The electrode pads (not shown) enable an electrical field to be set up across the bed (315). Preferably, the reactor bed (315) acts as a both a reactor and

as a separator.

A suitable μ -FFE microstructure for small scale reactions has a bed (215/315) that is 10 mm wide, 50 mm long, and 50 μ m in depth which is isolated from the electrodes (not shown) via two arrays of 2500 v-groove channels; platinum wire (not shown) - 30 μ m - for the electrodes which are placed into the device via holes in the cover plate (not shown); and wherein the beds (208/308 and 209/309) containing the electrodes are 2 mm wide, 50 mm long, and 50 μ m deep. Other suitable dimensions and materials may be used.

The general operation of a μ -FFE microstructure is explained in some detail in the above-mentioned papers by Raymond *et al.* However, Figure 8 presents diagrammatically the operation of such a device. In brief, in free-flow electrophoresis a narrow sample stream is continuously fed into a carrier solution which flows perpendicular to an applied electric field. Charged species are then deflected from the direction of flow at an angle determined by a combination of the carrier flow velocity and the respective electrophoretic mobilities of the sample components. The angle of deflection increases with the electric field strength and the mobility, and decreases with increasing carrier flow rate. In general, the large-scale preparative FFE systems either collect the individual sample bands and analyse these fractions off-line by UV/visible spectroscopy or incorporate some type of on-line monitoring.

With the present invention, however, more than one reactant (i.e. A and B) are fed into/onto the bed and moreover the bed itself serves a dual purpose. First, it is used to set up a reaction zone. Second, it is used to allow reacted and unreacted reactants to be separated. This particular operation of the μ -FFE microstructure according to the present invention is shown diagrammatically in Figures 9 and 10. In these Figures the reaction zones are indicated by arrows 400 and 500 respectively.

Thus, the present invention relates to a multi-step process.

A preferred embodiment of this aspect of the present invention is a multi-step process comprising the sequential steps of: (a) individually introducing reactant A1 and reactant B1 into a reaction zone R1; (b) allowing A1 and B1 to contact each other in the reaction zone R1; (c) individually removing from the reaction zone R1 any product P1 formed by the reaction of A1 with B1, any unreacted A1 and any unreacted B1; (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2; (e) allowing A2 and B2 to contact each other in the reaction zone R2; (f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2; wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time; wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time; and wherein the process is a combinatorial chemical process.

A more preferred embodiment of this aspect of the present invention is a multi-step process comprising the sequential steps of: (a) individually introducing reactant A1 and reactant B1 into a reaction zone R1; (b) allowing A1 and B1 to contact each other in the reaction zone R1; (c) individually removing from the reaction zone R1 any product P1 formed by the reaction of A1 with B1, any unreacted A1 and any unreacted B1; (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2; (e) allowing A2 and B2 to contact each other in the reaction zone R2; (f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2; wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time; wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time; wherein the process is a combinatorial chemical process; wherein the reactants A1, A2, B1, B2 are charged; and wherein the reactants are introduced into the reaction zone(s) by the use of a differential charge field.

A more preferred embodiment of this aspect of the present invention is a multi-step process comprising the sequential steps of: (a) individually introducing reactant A1 and reactant B1 into a reaction zone R1; (b) allowing A1 and B1 to contact each other in the reaction zone R1; (c) individually removing from the reaction zone R1 any

product P1 formed by the reaction of A1 with B1; any unreacted A1 and any unreacted B1; (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2; (e) allowing A2 and B2 to contact each other in the reaction zone R2; (f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2; wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time; wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time; wherein the process is a combinatorial chemical process; wherein the reactants A1, A2, B1, B2 are charged; wherein the reactants are introduced into the reaction zone(s) by the use of a differential charge field; and wherein the products and unreacted reactants are removed from the reaction zone(s) by the use of a differential charge field.

A highly preferred embodiment of this aspect of the present invention is a multi-step process comprising the sequential steps of: (a) individually introducing reactant A1 and reactant B1 into a reaction zone R1; (b) allowing A1 and B1 to contact each other in the reaction zone R1; (c) individually removing from the reaction zone R1 any product P1 formed by the reaction of A1 with B1, any unreacted A1 and any unreacted B1; (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2; (e) allowing A2 and B2 to contact each other in the reaction zone R2; (f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2; wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time; wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time; wherein the process is a combinatorial chemical process; wherein the reactants A1, A2, B1, B2 are charged; wherein the reactants are introduced into the reaction zone(s) by the use of a differential charge field; wherein the products and unreacted reactants are removed from the reaction zone(s) by the use of a differential charge field; and wherein any of product P1 and unreacted reactant A1 and B1 are have just left the reaction zone R1 and wherein reactants A2 and B2 are just about to enter the reaction zone R2.

In addition, the present invention relates to a device for use with the multi-step process of the present invention.

Especially preferred is the device when being used for the multi-step process of the present invention.

5 A preferred device according to the present invention comprises (i) a region providing reaction zone R1; (ii) a region providing reaction zone R2; (iii) an inlet for reactant A1 and an optional separate inlet for reactant A2; (iv) an inlet for reactant B1 and an optional separate inlet for reactant B2; (v) an outlet for unreacted reactant A1 and an optional separate outlet for unreacted reactant A2; (vi) an outlet for unreacted reactant B1 and an optional separate outlet for unreacted reactant B2; and (vii) an outlet for
10 any product P1 and an optional separate outlet for product P2; wherein the inlets and outlets are operably connected to the reaction zones; wherein there is a separate inlet for reactant A2 and/or a separate inlet for reactant B2; wherein there is a separate outlet for unreacted reactant A2 and/or a separate outlet for unreacted reactant B2; and wherein there is a separate outlet for product P2.

15 A more preferred device according to the present invention comprises (i) a region providing reaction zone R1; (ii) a region providing reaction zone R2; (iii) an inlet for reactant A1 and an optional separate inlet for reactant A2; (iv) an inlet for reactant B1 and an optional separate inlet for reactant B2; (v) an outlet for unreacted reactant
20 A1 and an optional separate outlet for unreacted reactant A2; (vi) an outlet for unreacted reactant B1 and an optional separate outlet for unreacted reactant B2; and (vii) an outlet for any product P1 and an optional separate outlet for product P2; wherein the inlets and outlets are operably connected to the reaction zones; wherein there is a separate inlet for reactant A2 and/or a separate inlet for reactant B2;
25 wherein there is a separate outlet for unreacted reactant A2 and/or a separate outlet for unreacted reactant B2; wherein there is a separate outlet for product P2; and wherein the device comprises means for delivering a differential charge field in order to introduce the reactants into the reaction zone(s).

30 A more preferred device according to the present invention comprises (i) a region providing reaction zone R1; (ii) a region providing reaction zone R2; (iii) an inlet for reactant A1 and an optional separate inlet for reactant A2; (iv) an inlet for reactant

B1 and an optional separate inlet for reactant B2; (v) an outlet for unreacted reactant A1 and an optional separate outlet for unreacted reactant A2; (vi) an outlet for unreacted reactant B1 and an optional separate outlet for unreacted reactant B2; and (vii) an outlet for any product P1 and an optional separate outlet for product P2; wherein the inlets and outlets are operably connected to the reaction zones; wherein there is a separate inlet for reactant A2 and/or a separate inlet for reactant B2; wherein there is a separate outlet for unreacted reactant A2 and/or a separate outlet for unreacted reactant B2; wherein there is a separate outlet for product P2; wherein the device comprises means for delivering a differential charge field in order to introduce the reactants into the reaction zone(s); and wherein the device comprises means for delivering a differential charge field in order to remove the products and unreacted reactants from the reaction zone(s).

A highly preferred device according to the present invention comprises (i) a region providing reaction zone R1; (ii) a region providing reaction zone R2; (iii) an inlet for reactant A1 and an optional separate inlet for reactant A2; (iv) an inlet for reactant B1 and an optional separate inlet for reactant B2; (v) an outlet for unreacted reactant A1 and an optional separate outlet for unreacted reactant A2; (vi) an outlet for unreacted reactant B1 and an optional separate outlet for unreacted reactant B2; and (vii) an outlet for any product P1 and an optional separate outlet for product P2; wherein the inlets and outlets are operably connected to the reaction zones; wherein there is a separate inlet for reactant A2 and/or a separate inlet for reactant B2; wherein there is a separate outlet for unreacted reactant A2 and/or a separate outlet for unreacted reactant B2; wherein there is a separate outlet for product P2; wherein the device comprises means for delivering a differential charge field in order to introduce the reactants into the reaction zone(s); wherein the device comprises means for delivering a differential charge field in order to remove the products and unreacted reactants from the reaction zone(s); and wherein any of product P1 and unreacted reactant A1 and B1 are in or on the device and have left the reaction zone R1 and wherein reactants A2 and B2 are in or on the device and are about to enter the reaction zone R2.

More preferred aspects of these embodiments is when the device is a wafer device, preferably a μ -FFE microstructure.

5 The present invention will now be described only by way of example, in which reference shall be made to Figures 1-10 which present schematic aspects of the present invention. Some aspects of these Figures have been discussed above.

10 As mentioned above, the layout of a device suitable for use in the present invention is shown in Figure 5 (taking into account the above commentary) or Figure 7. In order to prepare the device, micromachining techniques were used (IC Sensors Milpitas, CA) to obtain the silicon FFE devices. The channel systems were first etched into the silicon, after which a glass cover plate was anodically bonded to form the channels. The separation bed is 10 mm wide, 50 mm long, and 50 μ m deep, while the beds containing the electrodes are 2 mm wide, 50 mm long, and 50 μ m deep. In conventional FFE systems the separation bed is isolated from the beds containing the electrodes by use of a membrane spacer. This approach is not possible in the silicon device because the device is a closed unit, sealed by the cover glass plate. To achieve isolation of the separation bed from the electrode-containing beds, two arrays of 2500 v-groove channels (each groove is 12 μ m wide, 10 μ m deep, and 1 mm long) were incorporated to "act" as a membrane. Furthermore, the separation bed depth was controlled by the etch depth rather than by a spacer.

25 The inlet channel array consists of 125 grooves (each groove is 70 μ m wide, 50 μ m deep, and 5 mm long) and was used to prevent turbulent flow of the carrier buffer as it entered the separation bed. The identical outlet array was designed to be used as a fraction collector; however, in this device fractions could not be collected since only one outlet hole was fabricated. This was done to simplify fabrication for initial experimentation. To allow introduction of fluid into the device, inlet and outlet holes were etched through the silicon. Finally, in order to connect the device to the external fluid lines, a holder was constructed in-house from Plexiglas™ to which standard fittings and Teflon™ tubing were attached.

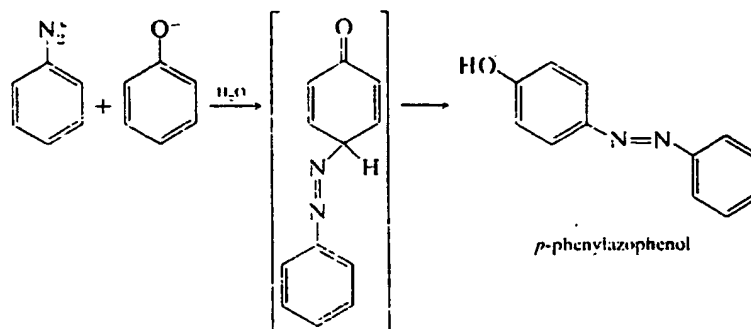
Electrodes were placed into the side beds *via* holes in the cover plate. The electrodes were obtained from 30 μm platinum wire which had been flattened with a hammer. To prevent the electrical current from flowing through the silicon device, an insulating layer consisting of 1000 Å of low-stress LCVD nitride was deposited onto n-type silicon (doping density of $10^{15}/\text{cm}^3$). This composite layer was expected to give a breakdown voltage for the silicon device of 100-200 V.

Table 1 provides a list of some working parameters for the present invention.

Instead of using a μ -FFE microstructure as mentioned above, the process of the present invention may be used with any other suitable structure or device. For example, other micromachining techniques could be used which employ TAS on glass and silicon, such as μ -TAS. Particular attention has been focused on the development of high-performance capillary electrophoresis on planar glass structures. Other structures include silicon wafer gas chromatography systems, liquid chromatography systems, flow injection analysis systems, absorbance detectors, flow type biosensors using immobilized enzymes or whole cells, and microfabricated reaction chambers. The incorporation of all sample handling and analysis on a single device, or stacks of devices, shows promise for medical, environmental, and biotechnological applications.

In order to show proof of principle of the process of the present invention a diazonium salt in a basic solution is fed into a first inlet and a phenol in a basic solution is fed into a second inlet of the device shown in Figure 7. A differential voltage field is then applied across the bed. Each of the reactants migrates to a reaction zone where they react together to form an arylazophenol. The product is then separated from the reactants in view of its neutral charge as it migrates to the outlet port. A typical reaction scheme is shown below.

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Other reactions may be performed.

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Thus, the present invention provides a time-encoded, sequential, multi-step, chemical reaction process.

Modifications of the present invention will be apparent to those skilled in the art.

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For example, instead of a electrical charge differential across the bed, a magnetic field could be applied.

CONTINUOUS FLOW ELECTROPHORETIC REACTOR for M. Smith, F. Schultz						REMARKS	
constants	diffusion	[m ² /s]	1.00E-09			for small ion	
	electrophoresis	[m ² /V/s]	1.00E-09			μ for single charged small ion	
reactor	length y	[m]	0.02	[mm]	20	y: direction of pumped flow	
	width x	[m]	0.02	[mm]	20	x: direction of electrophoresis	
	depth z	[m]	0.00005	[μ m]	50		
feed/exit	length y	[m]	0.001	[mm]	1		
channels	width x	[m]	0.00007	[μ m]	70		
	depth z	[m]	0.00005	[μ m]	50		
	no of channels		238			assuming 20% walls	
side channel	length x	[m]	0.00005	[μ m]	50		
array	width y	[m]	0.00001	[μ m]	10		
	depth z	[m]	0.000007	[μ m]	7		
	no of channels		1333			assuming 5 μ m walls	
resistances	reactor	[arb. units]	20,000				
	side channel array	[arb. units]	1,071			one side only	
	resistance ratio		18.6666667				
operating	applied voltage	[V]	200				
parameters	at reactor	[V]	181				
	migration +	[m/s]	9.0323E-06	[μ m/s]	9		
	transit time +	[s]	2214	[min]	36.9	100% of reactor	
	pumped flow +	[m ³ /s]	9.0323E-06	[μ m/s]	9	100% of reactor	
			9.0323E-12	[nL/min]	541.9		

	migration ++	[m/s]	1.8065E-05	[μm/s]	18	
	transit time ++	[s]	1107	[min]	18.45	
	pumped flow ++	[m/s]	1.8065E-05	[μm/s]	18	
		[m3/s]	1.8065E-11	[nL/min]	1084	
bandbroadening	reagent feed	[m]	0.00020207	[μm]	202	10 channels only, sigma
	diffusion +	[m]	0.00210442	[μm]	2104	sigma
	total bb +	[m]	0.0021141	[μm]	2114	sigma
	equivalent to		25.2	channels		
	diffusion ++	[m]	0.00148805	[μm]	1488	
	total bb ++	[m]	0.00150171	[μm]	1502	
	equivalent to		17.9	channels		
reaction site (+ only)	length y	[m]	0.00841767	[mm]	8	trapezoidal shape
	width x	[m]	0.00845639	[mm]	8	of 4 sigma area
	depth z	[m]	0.00005	[μm]	50	
	time	[s]	466	[min]	7.77	allowed for the reaction
	volume	[m3]	1.7796E-09	[μL]	1.78	
resolution of separation	distance n vs. +	[m]	0.01	[mm]	10	distance between conc. maxima
	4 sigma	[m]	0.00845639	[mm]	8	bandbroadening 4 sigma
	resolution		1.18253816			should be larger than 1

CLAIMS

1. A multi-step process comprising the sequential steps of:

5 (a) individually introducing reactant A1 and reactant B1 into a reaction zone R1;

(b) allowing A1 and B1 to contact each other in the reaction zone R1;

10 (c) individually removing from the reaction zone R1 any product P1 formed by the reaction of A1 with B1, any unreacted A1 and any unreacted B1;

15 (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2;

(e) allowing A2 and B2 to contact each other in the reaction zone R2;

20 (f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2;

wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time;
and

25 wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time.

2. A multi-step process according to claim 1 wherein the process is a combinatorial chemical process.

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3. A multi-step process according to any one of the preceding claims wherein the reactants A1 and/or A2 are charged.

4. A multi-step process according to any one of the preceding claims wherein the reactants A1 and A2 are charged.
5. A multi-step process according to any one of the preceding claims wherein the reactants B1 and/or B2 are charged.
6. A multi-step process according to any one of the preceding claims wherein the reactants B1 and B2 are charged.
7. A multi-step process according to any one of the preceding claims wherein the reaction zone is in or on a wafer device.
8. A multi-step process according to claim 7 wherein the wafer device is a μ -FFE microstructure.
9. A multi-step process according to any one of the preceding claims wherein the reactants are introduced into the reaction zone(s) by the use of a differential charge field.
10. A multi-step process according to any one of the preceding claims wherein the products and unreacted reactants are removed from the reaction zone(s) by the use of a differential charge field.
11. A compound when synthesised, analysed or screened by the multi-step process according to any one of claims 1 to 10.

12. A device for a multi-step process, which process comprises the sequential steps of:

(a) individually introducing reactant A1 and reactant B1 into a reaction zone R1;

5 (b) allowing A1 and B1 to contact each other in the reaction zone R1;

(c) individually removing from the reaction zone R1 any product P1 formed by the reaction of A1 with B1, any unreacted A1 and any unreacted B1;

10 (d) individually introducing reactant A2 and reactant B2 into a reaction zone R2;

(e) allowing A2 and B2 to contact each other in the reaction zone R2;

(f) individually removing from the reaction zone R2 any product P2 formed by the reaction of A2 with B2, any unreacted A2 and any unreacted B2;

15 wherein A1 and B1 are introduced into the reaction zone R1 at a predetermined time; and

wherein A2 and B2 are introduced into the reaction zone R2 at a predetermined time;

wherein the device comprises

20 (i) a region providing reaction zone R1;

(ii) a region providing reaction zone R2;

(iii) an inlet for reactant A1 and an optional separate inlet for reactant A2;

25 (iv) an inlet for reactant B1 and an optional separate inlet for reactant B2;

(v) an outlet for unreacted reactant A1 and an optional separate outlet for unreacted reactant A2;

(vi) an outlet for unreacted reactant B1 and an optional separate outlet for unreacted reactant B2; and

30 (vii) an outlet for any product P1 and an optional separate outlet for product P2;

wherein the inlets and outlets are operably connected to the reaction zones.

13. A device according to claim 12 wherein there is a separate inlet for reactant A2 and/or a separate inlet for reactant B2.

14. A device according to claim 12 or claim 13 wherein there is a separate outlet for unreacted reactant A2 and/or a separate outlet for unreacted reactant B2.

15. A device according to any one of claims 12 to 14 wherein there is a separate outlet for product P2.

16. A device according to any one of claims 12 to 15 wherein the device is a wafer device.

17. A device according to claim 16 wherein the wafer device is a μ -FFE microstructure.

18. A device according to any one of claims 12 to 17 wherein the device comprises means for delivering a differential charge field in order to introduce the reactants into the reaction zone(s).

19. A device according to any one of claims 12 to 18 wherein the device comprises means for delivering a differential charge field in order to remove the products and unreacted reactants from the reaction zone(s).

20. A device according to any one of claims 12 to 19 wherein any of product P1 and unreacted reactant A1 and B1 are in or on the device and have left the reaction zone R1 and wherein reactants A2 and B2 are in or on the device and are about to enter the reaction zone R2.

21. A time-encoded, sequential, multi-step, chemical reaction process.

22. A method of performing combinatorial chemistry in which reactants are introduced in to a reaction zone by means of a charge field, and the products of the reaction are subjected to a charge field to separate the products.

5 23. A process as depicted in any one or more of Figures 1-4, 7, 9 and 10.

24. A device as depicted in any one or more of Figures 4, 7, 9 and 10.

25. A process substantially as described herein.

10

26. A device substantially as described herein.



Application No: GB 9624871.1
Claims searched: 1-11

Examiner: Diane Davies
Date of search: 15 July 1997

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK CI (Ed.O): C2C: CMA

Int CI (Ed.6): C07C 245/08

Other: Online: CAS-ONLINE, EDOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X,Y	WO 9607917 A (Nanogen) Whole document: combinatorial processes using a microelectronic system for carrying out multi-step and multiplex reactions and using FFE to transport charged species to reaction zones (26).	1-11
Y	Anal. Chem., 66, 2858-65, (1994) D.E. Raymond <i>et al</i> , "Continuous sample pretreatment using a free-flow electrophoresis device integrated onto a silicon chip"	1-11
X,Y	Chemistry in Britain, 31-33, (1996) D. Craston & S. Cowen, "Processing on a chip": see in particular, paragraphs headed "Speeding ahead" suggesting use of μ -TAS for chemical reactions.	1-11

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.